2173-Pos Board B159
Chromatin Ordering in the SV40 Virus
Gadiel Saper, Stanislav Kler, Ariella Oppenheim, Uri Raviv, **Daniel Harries**.
The Simian Virus 40 (SV40) is composed of an outer shell formed from capsid proteins, enveloping a minichromosome that is made of double stranded DNA wound around ca. 20 nucleosomes. In contrast to many bacteriophages and RNA viruses, the structure and order of the packaged viral chromatin has remained elusive. Using small angle x-ray diffraction as well as computer modeling, we show that a unique ordering of the nucleic acid emerges, indicating at least two concentric shells of higher minichromosomal electron density. Analysis shows that packaging can be explained by considering the competition of interactions between disk-like nucleosomal particles that favor columnar ordering versus wall-nucleosome interactions that tend to align particles with the capsid interior.

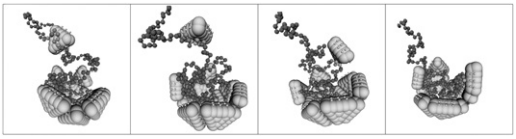
2174-Pos Board B160
Assembly of the Adenoviral IVa2 and L4-22K Proteins on a Viral DNA Packaging Sequence
Teng-Chieh (Jay) Yang.
A critical step in the viral life cycle of ds DNA viruses such as Adenovirus, Bacteriophage lambda and Herpes, is the encapsidation of the viral genome. In human Adenovirus, this step is initiated by the assembly of two viral proteins, called IVa2 and L4-22K, onto conserved sequences within the viral genome. Genetic studies have shown that a critical feature of these sequences is that multiple copies are present, which strongly suggests that heterotropic cooperative interactions between these two proteins control the viral decision to initiate the genome encapsidation reaction. Precise control of the viruses decision to begin to manufacture viral particles is required to optimize virus particle numbers. Here we apply rigorous hydrodynamic and thermodynamic approaches to investigate the equilibrium mechanism of assembly onto viral DNA. Based on these data, we propose a detailed biochemical model which provides, for the first time, a predictive understanding of the regulation of viral DNA packaging.

2175-Pos Board B161
Assembly of an Unenveloped Icosahedral RNA Viruses using Coarse-Grained Models
Mustafa B. Boz, Thanawadee Preeprem, Stephen C. Harvey.
We investigate the assembly of Satellite Tobacco Mosaic Virus (STMV) in a coarse-grained model. We use multi-level coarse-grained representations to decrease the computational expenses and adequately represent the different parts of the viral structure. The RNA coarse grain model was generated from a combination of an idealized RNA secondary structure based on the X-ray crystal and a proposed tRNA-like secondary structure at the 3' end. The RNA model has one pseudo atom (bead) per residue. The coarse-grained model for the capsid contains 20 triangular units, each of which also contains three flexible positively charged protein tails. The assembly process as well as the stability of the virus mainly depends on RNA-protein and protein-protein interactions. The protein tails are attracted to the RNA by electrostatic interactions while the capsid proteins are weakly attracted with each other by hydrophobic interactions. We modeled RNA-protein interactions with a Debye-Hückel potential and protein-protein interaction with a Lennard-Jones potential. We varied values of these two interactions to find regions where the virus is stable and will self-assemble, and construct a phase diagram of viral stability. Finally we investigated the assembly of the virus using molecular dynamics. These simulations help us understand the individual roles of these two interactions on viral assembly.

2176-Pos Board B162
Computational Modeling of DNA Ejection from Bacteriophages to Bacterial Cells
Anton S. Petrov, Stephen C. Harvey.
Ejection of DNA from bacteriophages to bacterial cells is a spontaneous process (at least in the initial stage) and does not require the aid of an external motor. However, packaging experiments and simulations predict that the internal pressure drops to nearly zero if the amount of DNA inside the bacteriophage is less than 50%. Thus, the internal pressure itself is believed to be incapable of driving the ejection of the remaining genome.
We developed a coarse-grained model to study DNA ejection, which includes DNA represented by beads on a string, a capsid treated as a spherical constraint, a channel, and a bacterial cell modeled as a large sphere. The ejection simulations were carried out using a Langevin Dynamics protocol to account for the viscosity of the medium inside the bacterial cell. Additionally, we applied a local force to mimic the osmotic pressure inside the cell. The model of DNA ejection is further improved to account for the explicit presence of macromolecules inside bacterial cells.
We found that in all cases the ejection force drops to nearly zero as the initial fraction of 50%-60% of DNA is ejected. However, at low viscosity and/or low (or zero) osmotic pressure, the force increases up to a few piconewtons as the remaining fraction of DNA is ejected. This additional force is due to the conformational constraints of the ejected DNA. The force increase is not seen in case of large viscosity and/or applied pressure of 2-4 atm. Explicit crowding agents inside the cell affected both the thermodynamics and kinetics of ejection: because only a certain volume fraction was available for DNA, the ejection force was found to be smaller compared to the case with continuum viscous media. Smaller force resulted in longer ejection times.

2177-Pos Board B163
Order Parameters for Multiscale Simulation of Bio-Nanosystems
Abhishek Singharoy, Peter Ortoleva.
Order Parameters (OPs) characterizing the structure and organization of bio-nanosystems (BNSs) are presented. Deductive all-atom multiscale techniques imply that structural transitions in several BNSs can be simulated via the slow, temporal OP dynamics, which co-evolves with a quasi-equilibrium probability density for rapidly fluctuating atomic configurations. This yields a force-field based algorithm that allows for all-atom BNS simulations with high CPU efficiency. Salient features of a set of OPs including their construction, emergence and dynamical completeness are discussed. These are critical in probing the free-energy landscapes underlying structural transitions. The computational algorithm is implemented via a software, denoted SimNanoWorld, that we demonstrate in applications to macromolecules and macromolecular assemblies. This includes simulating the dynamics of viral RNA and RNA-protein complexes in Satellite Tobacco Mosaic Virus (STMV) over a range of electrolytes (1:1 and 2:1) and temperatures (300K-600K). Another example demonstrates the application of OPs in probing the stability and immunogenicity of a T =1 Human Papillomavirus (HPV) L1-protein Virus-like Particle (VLP). Prospects of computer-aided vaccine design will be discussed.

2178-Pos Board B164
Dynamic Encapsidation of a Flexible Polymer by an Icosahedral Virus
Oren M. Elrad, Michael F. Hagan.
The coat proteins of many viruses spontaneously form icosahedral capsids around nucleic acids or other polymers, but the mechanism of this encapsulation process is incompletely understood. Elucidating the role of the packaged polymer in capsid formation could promote biomedical efforts to block viral replication and enable use of capsids in nanomaterials applications. To this end, we perform Brownian dynamics on a coarse-grained model that describes the assembly of an icosahedral capsid around a flexible polymer. The simulations enable experimentally testable predictions for the results of the assembly reaction as a function of experimentally accessible parameters such as polymer length, polymer-protein stoichiometry, and solution conditions. Several of the capsid morphologies that assemble around longer than optimal polymers resemble structures which have been seen experimentally. Furthermore, the simulations demonstrate that experimental control parameters dictate the mechanism by which assembly occurs. Under some conditions the polymer actively promotes its encapsulation through cooperative polymer-protein motions, resulting in an assembly mechanism entirely unlike those seen for empty capsid assembly. We also explore the role of polymer secondary structure on assembly efficiency.



2179-Pos Board B165
Learning Physical Parameters of Capsid Assembly Systems from Indirect Measures of Assembly Progress
M. Senthil Kumar, Gregory Smith, Lu Xie, Rupinder P. Khandpur, **Russell Schwartz**.
Theoretical models and simulation methods have played important roles in understanding virus capsid assembly, providing key insights into possible modes and pathways of assembly, the physical mechanisms by which these are controlled, and potential sources of off-pathway assembly and the means by which they may be avoided. These theoretical and simulation studies have, however, traditionally been limited in their ability to draw conclusions about specific capsid assembly systems, largely because the theoretical models are controlled by physical parameters (e.g., binding rates), that cannot be analytically determined from any available experimental data source. Much of the seminal work on theoretical models of capsid assembly has therefore been confined either to looking at highly simplified models of

generic icosahedral capsid assembly or to studies of ranges of possibilities found over broad parameter domains. We describe work intended to help bridge this gap between theoretical models of capsid assembly in general and experimental work on specific model systems by using computational parameter estimation to learn rate parameters for stochastic simulations of capsid assembly from available experimental data. Our method combines ideas from gradient-based and response-surface optimization methods with a heuristic global search strategy to find parameter fits that approximately reproduce experimental measures of overall assembly progress. We demonstrate the approach through application to light scattering data tracking assembly progress of several in vitro capsid assembly systems. The results provide insight into possible mechanisms and pathways of assembly for specific capsid systems in vitro. They further provide a basis for future studies attempting to computationally project how behavior of these systems would be altered in conditions more closely approximating those expected at sites of capsid assembly in vivo.

2180-Pos Board B166

Coarse-Grained Molecular Dynamics Simulations of the Entire Influenza Virus Envelope

Daniel L. Parton, Marc Baaden, Mark S.P. Sansom.

The envelope of the influenza virus contains three membrane proteins: hemagglutinin (HA), neuraminidase (NA) and the M2 proton channel. The interactions of these proteins with their surrounding lipid environment are important for many phases of the viral life cycle. In the various membranes of an infected host cell, newly formed viral proteins are thought to use lipid rafts - small patches of ordered membrane - to locate themselves at the plasma membrane. The arrangement of the proteins within the envelope of free virions may also be important for the infectivity of the virus.

We have used the MARTINI coarse-grained force field to simulate a viral envelope of realistic size for several microseconds. Coarse-grained methods allow simulations on large systems (4.5 million particles for the system in this work) over extended timescales. Using information from recent cryo-electron tomography images of complete virions as a basis, our model has been constructed as a 60 nm diameter lipid vesicle with 80 HA, 12 NA and 12 M2 proteins inserted in the membrane. The protein structures are derived from existing crystallographic and NMR structures. The vesicle membrane is a ternary mixture of saturated and poly-unsaturated phospholipids, and cholesterol, which has been shown in other work to separate into raft and non-raft phases.

The simulations will be analysed to provide information on the structural and dynamical properties of the viral envelope. In particular, we will focus on the partitioning of proteins between raft and non-raft lipid domains, and the degree of protein clustering.

2181-Pos Board B167

Reducing Immune Response against Lentiviral Vectors: Lentiviral Vector Presentation of CD47, The 'Marker of Self'

Nisha Sosale, Richard K. Tsai, Irena Ivanovska, Philip W. Zoltick, Dennis E. Discher.

Immune response to viral gene therapy vectors and their transgene products is a significant problem in the field of gene therapy. Viral vectors, because they are derived from viruses, can induce an immune response. This makes gene delivery inefficient and can pose a significant danger to patients. Macrophages act as immunological gatekeepers at the interface of tissue and lymph. They take up antigens from the extracellular environment and then present them to the immune system. Macrophage uptake has been shown to be inhibited by CD47 interaction with SIRP alpha. Viral vectors presenting CD47 on their surface should show reduced levels of phagocytosis by macrophages, and thus reduced presentation and clearance by the immune system. In this work, HEK 293T cells were transduced, using a lentiviral vector, to over-express CD47 with green fluorescent protein (GFP) at the C-terminus. These transduced cells were then transfected to produce a second set of lentiviral vectors. Since the lentivirus takes a piece of the cell membrane to make its envelope when it buds from the cell, these vectors express CD47 on their envelope. The main goal of this work is to qualitatively and quantitatively characterize the presentation of CD47 by these lentiviral vectors. Fluorescent microscopy of equilibrium density gradient fractions indicates that these lentiviral vectors present CD47-GFP. The fluorescence intensity of individual and aggregated viral vectors was quantified. This will be the first step in using CD47 expression as a method to reduce immune response to lentiviral vectors in order to increase the efficacy and safety of lentiviral vector mediated gene therapy.

2182-Pos Board B168

In Vivo and In Vitro Assembly of Sindbis Virus Nucleocapsid-Defective Mutants

Christian Berrios, Jonathan Snyder, Joyce Jose, Richard Kuhn.

Sindbis virus (SINV) is a member of the Togaviridae family, in the alphavirus subfamily. Alphaviruses are arthropod-borne spherical, enveloped, positive-sense single-stranded RNA viruses. They cause a variety of human and animal diseases, ranging from fever and rash to encephalitis. The SINV nucleocapsid (NC) contains the RNA genome, and is composed of 240 copies of a single 264-amino-acid capsid protein (CP). The process of NC assembly is poorly understood. However, the assembly process can be recapitulated *in vitro* using purified SINV capsid protein and single-stranded nucleic acids. Two specific lysine residues, K250 and K252, on the CP have been implicated in the assembly process of NC *in vivo.* Four CP mutants were developed that have had these two lysine residues either deleted, or changed to aspartic acid, glutamic acid, or alanine. We have shown that the glutamic acid and alanine mutants assemble at standard *in vitro* NC assembly conditions. In mammalian cells, these CP mutants expressed in the context of a viral infection lead to an attenuated phenotype, including lack of NC formation. However, virus infection in mosquito cells or expression of CP alone in mammalian cells leads to NC accumulation, like wild-type virus. We continue to pursue the disparity between *in vivo* and *in vitro* assembly results, relating them to the importance of these residues in the NC assembly process. These results could lead to a better understanding of the alphavirus NC assembly process and provide putative drug targets.

2183-Pos Board B169

Cooperative Assembly of Host and Viral Proteins into a DNA Packaging Motor Complex Analyzed by Fluorescence-Monitored Analytical Ultracentrifugation

Saurarshi Sanyal, Carlos E. Catalano.

Packaging of viral genomes into procapsids by ***terminase***enzymes is conserved in complex double-stranded DNA viruses. Terminases bind to linear concatemers of replicated viral genomes linked end-to-end and concomitantly excise (mature) and translocate (package) a single genome per procapsid. While the terminase maturation complex requires site-specific recognition to mature the genome ends at each *cos* site within the concatemer, the terminase motor complex must bind DNA tightly, but non-specifically, during packaging. The bacteriophage λ protomer is a heterotrimer composed of two different subunits that separately confer catalytic activities and site-specific DNA binding. Although our data demonstrate that the protomer self-associates into a ring-like tetramer, the stoichiometries of terminase in the maturation and packaging complexes remains unknown. We investigated the nature of the terminase-DNA maturation complex and the factors that mediate site-specific vs. non-specific DNA binding modes. Specifically, we utilized electrophoretic mobility shift assays (**EMSA**) and analytical ultracentrifugation (**AUC**) to examine assembly of λ terminase and *E. coli* Integration Host Factor (***IHF***, required for λ development *in vivo*) on model DNA substrates. We first demonstrate that terminase protomer does not significantly discriminate between *cos*-containing and non-specific DNA substrates using EMSA; however, in the presence of IHF the enzyme forms specific complexes with *cos*-DNA at concentrations much lower than those required to bind non-specific DNA. We next utilize AUC to demonstrate that a binary complex is formed between *cos*-DNA and IHF. Addition of λ terminase to this binary complex affords two higher-order ternary complexes in a concentration-dependent manner. The data demonstrate that IHF promotes cooperative and site-specific assembly of terminase at the packaging initiation site *cos*. The nature of the nucleoprotein complexes will be discussed in relation to their role in viral genome packaging.

2184-Pos Board B170

Characterization of the Interaction of the Dengue Virus Capsid Protein with Lipid Droplets

Ivo C. Martins, Fabiana A. Carneiro, Ronaldo Mohana-Borges, André Faustino, Renata M. Pereira, Filomena A. Carvalho, Miguel A. Castanho, Fábio Almeida, Nuno C. Santos, Andrea T. DaPoian.

Dengue virus affects 100 million people yearly, but this number may grow since *Aedes* spp. mosquitoes, the disease vectors, are spreading to temperate climates, including in the USA. No effective vaccines are available. A poor understanding of the viral life cycle is to blame, especially regarding the viral assembly and encapsidation process, mediated by Dengue Virus Capsid Protein (DVCP). DVCP is a symmetric homodimmer α-helical protein that must interact with intracellular lipid droplets during viral encapsidation. DVCP charge distribution suggests that its *α2-α2'* nonpolar region may interact with lipids and the *α4-α4'* positive charged region could interact with viral RNA. By employing biophysical techniques combined with bioinformatics tools, we found this hypothesis correct.